Some Effects of Organic Growth-Promoting Substances (Auximones) on the Growth of Lemna minor *in Mineral Culture Solutions.*

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[Plates 21 and 22.]

Introduction.

In a previous communication* experiments were described which indicated that when peat is incubated with a mixed culture of aërobic soil organisms for about fourteen days at a temperature of 26° C., a rapid decomposition of the organic matter takes place, with the formation in the "bacterised" peat of certain organic growth-promoting substances or "auximones,"† the addition of which in very small amounts to wheat seedlings growing in water culture causes a marked increase in growth. It was desirable to repeat these experiments with plants in which any variation in growth could be readily and more accurately estimated than in wheat seedlings.

There was a difficulty at first in selecting a suitable plant for experiment. The objections to using the seedlings of land plants are: the difficulty of accurate weighings at regular intervals; the fact that a water culture solution is not the natural habitat for a land plant; the possibility that such seedlings may contain a supply of organic growth-promoting substances produced from the endosperm during germination. Water plants, on the other hand, are usually considered unsuitable for water culture experiments because they will not grow for any length of time in pure mineral culture solutions. Darwin and Acton‡ state that "water plants cannot generally be recommended for accurate experiments extending over any considerable time, as we have found it much more difficult to grow them satisfactorily in culture solutions than to grow ordinary plants with the roots immersed." They say, however, "we have found *Lemna minor* useful for purposes of demonstration. They grow rapidly, and their increase being principally in one plane is easily noticed at a glance. Moreover a rough numerical estimate of the amount of increase in a given time can be made by counting the fronds."

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* 'Roy. Soc. Proc.,' B, vol. 88, pp. 237–247 (1914).

† In a previous communication ('Roy. Soc. Proc.,' B, vol. 89, p. 102 (1915)) the term "auximone" (Gr. αὔξιμος: promoting growth) has been suggested for these substances. This term may usefully serve as a general descriptive name for these organic plant growth-promoting substances until our knowledge of their true nature and composition is sufficiently extended to warrant the application of a more satisfactory name.

‡ Darwin and Acton, 'Practical Physiology of Plants,' pp. 61–63 (1901).

A preliminary experiment with *Lemna minor* growing in water culture solutions showed that this plant responds readily to the presence of auximones, and, when supplied with these substances, there is strong and healthy growth for an indefinite period. Also by counting the number and measuring the areas of the fronds at regular intervals of time when growing in different culture solutions, a reliable estimate of variations of growth can be obtained.

Accordingly a detailed investigation of the influence of certain auximones on the growth of *Lemna minor* was commenced. On July 1, 1915, three similar porcelain dishes of 1 litre capacity were prepared: one containing 600 c.c. of Detmer's complete culture solution made up with glass-distilled water; the second, the same amount of Detmer's solution with the addition of the decomposed phosphotungstic acid fraction of bacterised peat, extracted as described in the previous communication, in the proportion of 17 parts of dry substance per million of solution; and the third, Detmer's solution plus the silver-baryta fraction of bacterised peat, in the proportion of 0·35 part per million.

Thirty healthy plants of *Lemna minor* of uniform size were counted out into each of the three dishes. The dishes were then covered with glass plates to exclude dust, and were kept in a cool greenhouse. At the end of a week the number of plants in each dish was again counted, their area measured by means of squared paper, and the liquids replaced by fresh solutions. This treatment was repeated weekly, with slight irregularities, until October 20.

At the end of six weeks the plants receiving the phosphotungstic fraction had filled their dish, therefore the contents of the three dishes were divided exactly into half at the weekly counting, and one-half of each set was discarded. This had to be repeated frequently owing to the very rapid multiplication of the auximone plants, until on October 20, when the experiment was concluded, the number of plants in the respective dishes had then to be multiplied by 256, to obtain the total numbers which would have been derived from the original 30.

The final figures obtained showed a percentage increase in number over the Detmer's solution of 197 for the phosphotungstic fraction, and 109 for the silver fraction.

The plants receiving auximones retained their vigour and maintained their original size of 3·73 sq. mm. throughout the experiment, whilst the control plants soon began to show signs of starvation, and decreased in size week by week, until at the end of the experiment they had diminished from 3·73 to 0·78 sq. mm., and it became difficult to count and measure them accurately.

Microscopic investigation also showed striking differences in internal structure. In the control plants there was an excessive number of large air spaces. The individual cells were small, with a large central vacuole and a small nucleus. In the auximone plants the tissues were more compact, with fewer and smaller air spaces. The large cells were filled with cytoplasm and possessed a well-developed nucleus which stained deeply.

In these experiments there was only one dish of plants for each variation of culture solution, and it was considered that "the result could not be regarded as conclusive, owing to the probability of experimental errors." It was therefore necessary to repeat and extend the experiments with a larger number of dishes for each culture solution. After consultation with certain botanical colleagues, it was decided that each set should consist of 10 dishes, that the rate of growth should be estimated by (1) increase in number of plants, (2) increase of dry weight, instead of measurement, each time the sets were halved, and that other extracts of bacterised peat should be tested.

Owing to the lack of suitable greenhouse accommodation at King's College for these experiments, the authorities of the Botanical Department of the Imperial College of Science and Technology, South Kensington, kindly granted permission and offered facilities for the work to be carried out in the greenhouse laboratory of that college.

Experiments with Ordinary Distilled Water.

On June 9 last year, five series, each consisting of 10 dishes, were prepared, and were numbered from 1 to 50. Flat-bottomed glass crystallising dishes of 4 inches diameter were employed, all containing 250 c.c. of the required solution. Each of the dishes was then enveloped on the outside, to the level of the contained liquid, with paper which was dull black on one side and white on the other, the black side being towards the dish and the white towards the exterior, in order to prevent as much as possible access of any heat and light rays through the sides and bottom of the vessels.

The solutions employed were:—Series I, Detmer's standard culture solution; Series II, Detmer's solution, together with a water extract of bacterised peat; Series III, Detmer's solution, with a similar extract freed from humic acid; Series IV, Detmer's solution, plus an alcoholic extract of bacterised peat; Series V, Detmer's solution, with the addition of the phosphotungstic acid fraction of bacterised peat. No experiments were made with the silver fraction on account of the lack of available material at the commencement of the experiment.

Detmer's solution was the standard solution consisting of potassium nitrate, 7 grm.; dipotassium phosphate, 1·5 grm.; magnesium sulphate, 1·5 grm.; sodium chloride, 1·5 grm.; calcium sulphate in excess (5 grm. were used); ferric chloride solution, a few drops; and distilled water, 3000 c.c. This solution contained nitrogen, phosphorus, and potash, estimated as NH_3, P_2O_5, and K_2O, in the proportion of 393, 204, and 1220 parts per million respectively, the total concentration of salts being about 5500 parts per million.

The water extract of bacterised peat was prepared by leaching out, by means of boiling distilled water, all soluble matter from a weighed quantity of bacterised peat, and making up the liquid to a known volume. An aliquot portion of this solution, containing the extract from ½ grm. bacterised peat, estimated on the dry weight, was added to each of the dishes containing Detmer's solution in Series II.

The water extract of bacterised peat, freed from humic acid, was prepared by precipitating the humic acid from a water extract prepared as above, by the requisite amount of a very dilute solution of calcium chloride, and removing the calcium humate by filtration. An aliquot portion of this liquid, representing the extract from ½ grm. peat, was added to each of the dishes in Series III.

The alcoholic extract and the phosphotungstic fraction were obtained by the methods described in the previous communication. The alcoholic extract from 1 grm. of bacterised peat was added to each of the dishes in Series IV, and the phosphotungstic fraction from 2½ grm. to each dish in Series V.

In order to ensure the use of uniform culture solutions, stock solutions of all the required substances, sufficient to last throughout the whole of the experiment, were prepared at the outset. The Detmer's culture solution was prepared in a concentration of 100 times the strength required, and the various organic extracts were prepared in concentrated solutions, to which a little chloroform water was added to prevent bacterial action. The chloroform was removed by gentle evaporation on the water-bath each time the liquids were required for use.

The proportions of total substances added in the various series to the 5500 parts per million of mineral salts in the Detmer's solution are shown in the Table below.

Table I.

Series.	Total solids added (parts per million).	Organic substance (parts per million).	Inorganic matter (parts per million).
II	421	368	53
III	189	97	92
IV	44	32	12
V	17	13	4

The greater quantity of inorganic matter in Series III than in Series II is explained by the substitution of the inorganic chloride for the organic humate radicle, and possibly also by a slight excess of calcium chloride used.

An estimation of the mineral nutrients, nitrogen, phosphorus and potash, was made only for Series II, as this series contained the maximum addition of these nutrients. The figures obtained for these additions, as compared with the quantities already present in Detmer's solution, are as follows :—

Table II.

	Amount of nutrient present in Detmer (parts per million).	Amount of same nutrient added (parts per million).	Percentage of addition.
Nitrogen (estimated as NH_3)	393	19·2	4·8
Phosphorus (estimated as P_2O_5)	204	3·6	1·8
Potassium (estimated as K_2O)	1220	0·52	0·04

Twenty plants of *Lemna minor*, as nearly uniform as possible in size, general healthiness and root development, were counted out into each of the 50 dishes. The solutions were changed twice each week in order to maintain, as nearly as possible, the original balance of the salts, and to eliminate bacterial contamination ; and, at each changing, the dishes were thoroughly cleansed before the solutions were renewed. The plants in each dish were counted every week.

The plants in all the series multiplied fairly uniformly for the 1st week, then the effect of the auximones in Series II and III became very marked, until at the end of three weeks the plants in these series completely filled their dishes. At this stage the plants in all 50 dishes were halved, one half being retained, and the dry weight of the other half estimated. This was repeated for a further three weeks, but at the end of the 7th week the plants in Series II and III had become so numerous that they had to be quartered, one-quarter only being retained instead of one-half.

Table III.

Series No.	Set No.	Beginning of expt.	1st week.	2nd week.	3rd week.		4th week.		5th week.		6th week.		7th week.	8th week.
		No. of plants.	No.	No.	No.	Wt.	No.	Wt.	No.	Wt.	No.	Wt.	No.	No.
						mgrm.		mgrm.		mgrm.		mgrm.		
I	1	20	31	49	82	10 ·0	148	10 ·4	208	8 ·0	336	12 ·8	608	1,120
	2	20	30	51	74	8 ·4	140	8 ·8	184	8 ·0	304	12 ·8	544	1,152
	3	20	31	52	78	11·6	128	12 ·8	192	14 ·4	304	19 ·2	576	1,056
	4	20	26	44	68	10 ·0	112	12 ·0	168	12 ·8	288	19 ·2	416	768
	5	20	30	55	74	8 ·8	140	9 ·6	248	11 ·2	352	19 ·2	640	1,120
	6	20	31	55	88	11 ·2	144	14 ·4	232	16 ·0	400	22 ·4	640	1,312
	7	20	31	56	72	9 ·6	148	8 ·0	200	14 ·4	288	19 ·2	416	896
	8	20	34	56	82	11 ·2	140	16 ·0	264	12 ·8	336	16 ·0	476	1,056
	9	20	31	57	84	10 ·8	140	14 ·4	240	14 ·4	352	22 ·4	608	1,184
	10	20	28	47	64	9 ·2	112	12 ·8	176	12 ·8	304	12 ·8	480	864
	Mean	20	30 ·3	52 ·2	76 ·6	10 ·1	135 ·2	11 ·9	211 ·2	12 ·5	326 ·4	17 ·6	550 ·4	1,052 ·8
II	11	20	41	98	326	55 ·2	1,104	185 ·6	3,016	483 ·2	7,312	1,161 ·6	20,032	77,312
	12	20	36	97	324	47 ·6	1,128	192 ·0	3,272	515 ·2	7,056	1,155 ·2	19,904	74,496
	13	20	36	108	340	54 ·0	1,108	117 ·6	3,064	491 ·2	6,144	1,049 ·6	20,096	57,856
	14	20	37	100	290	45 ·2	980	169 ·6	2,728	484 ·8	6,032	976 ·0	18,880	68,352
	15	20	37	96	292	56 ·4	920	133 ·6	2,568	441 ·6	5,712	918 ·4	18,624	62,464
	16	20	33	93	302	54 ·4	1,032	188 ·0	2,960	529 ·6	7,040	1,206 ·4	19,328	74,752
	17	20	40	111	382	63 ·6	1,300	208 ·8	3,408	587 ·2	7,888	1,283 ·2	20,800	62,208
	18	20	37	110	366	58 ·0	1,308	219 ·2	3,696	580 ·8	7,312	1,139 ·2	21,696	75,776
	19	20	39	101	320	53 ·6	1,044	162 ·4	2,816	436 ·8	6,128	1,056 ·0	17,216	65,024
	20	20	39	107	322	48 ·8	1,080	174 ·4	3,120	566 ·4	6,608	1,084 ·8	21,056	75,264
	Mean	20	37 ·5	102 ·1	326 ·4	53 ·7	1,100 ·4	181 ·1	3,064 ·8	511 ·7	6,723 ·2	1,103 ·0	19,763 ·2	69,350 ·4

III	21	20	36	77	218	30 ·0	576	87 ·2	1,320	244 ·8	3,120	492 ·8	9,472	35,456
	22	20	38	77	206	28 ·4	568	84 ·0	1,360	193 ·6	2,992	505 ·6	9,408	38,272
	23	20	35	76	176	22 ·8	448	58 ·4	1,232	177 ·6	2,768	448 ·0	8,512	35,968
	24	20	37	80	204	28 ·8	528	77 ·6	1,392	203 ·2	2,944	499 ·2	9,856	38,144
	25	20	38	82	202	30 ·8	544	86 ·4	1,464	222 ·4	3,104	553 ·6	10,560	42,624
	26	20	32	74	214	31 ·6	616	92 ·8	1,544	240 ·0	3,504	588 ·8	10,688	39,936
	27	20	34	70	188	28 ·0	476	68 ·8	1,216	172 ·8	2,944	441 ·6	8,576	38,912
	28	20	36	81	228	37 ·6	788	122 ·4	1,664	238 ·4	3,760	652 ·8	10,112	41,856
	29	20	35	70	210	28 ·4	608	82 ·4	1,544	256 ·0	3,536	582 ·4	11,776	42,368
	30	20	34	72	164	22 ·4	460	63 ·2	1,216	187 ·2	2,576	400 ·0	8,704	34,304
	Mean	20	35 ·5	75 ·9	210 ·0	28 ·9	561 ·2	82 ·3	1,395 ·2	213 ·6	3,124 ·8	516 ·5	9,766 ·4	38,784
IV	31	20	35	63	124	20 ·4	256	26 ·4	504	65 ·6	1,040	108 ·8	3,040	12,608
	32	20	34	55	110	14 ·0	304	23 ·2	584	65 ·6	1,136	137 ·6	3,136	14,336
	33	20	33	61	132	18 ·4	304	34 ·4	576	62 ·4	1,216	140 ·8	3,264	14,080
	34	20	32	60	114	14 ·8	284	24 ·8	488	44 ·8	992	105 ·6	2,848	11,776
	35	20	36	63	132	17 ·2	312	37 ·6	656	78 ·4	1,328	166 ·4	4,544	16,064
	36	20	35	66	142	21 ·2	304	42 ·4	680	83 ·2	1,504	204 ·8	5,184	15,744
	37	20	31	53	104	13 ·2	224	21 ·6	464	44 ·8	912	102 ·4	2,336	10,496
	38	20	33	57	122	14 ·0	252	28 ·8	432	46 ·4	800	99 ·2	2,144	10,752
	39	20	35	60	126	17 ·6	288	33 ·6	528	59 ·2	1,024	118 ·4	2,880	12,416
	40	20	29	58	112	16 ·0	276	31 ·2	512	64 ·0	1,056	112 ·0	2,592	11,520
	Mean	20	33 ·3	59 ·6	121 ·8	16 ·7	280 ·4	30 ·4	542 ·4	61 ·4	1,100 ·8	129 ·6	3,196 ·8	12,979 ·2
V	41	20	37	62	96	12 ·4	184	20 ·8	312	24 ·0	512	54 ·4	864	1,984
	42	20	34	52	90	12 ·8	152	14 ·4	272	27 ·2	448	44 ·8	928	2,016
	43	20	35	56	96	12 ·0	168	18 ·4	256	24 ·0	416	38 ·4	832	1,504
	44	20	33	62	96	11 ·6	176	20 ·8	272	25 ·6	448	41 ·6	832	1,920
	45	20	30	55	86	10 ·0	168	19 ·2	280	30 ·4	464	48 ·0	1,024	2,656
	46	20	34	61	112	15 ·6	200	24 ·8	328	32 ·0	592	51 ·2	1,376	3,104
	47	20	32	55	92	12 ·8	176	23 ·2	296	27 ·2	528	38 ·4	1,056	2,496
	48	20	29	50	86	12 ·4	152	17 ·6	272	30 ·4	448	48 ·0	960	2,016
	49	20	33	55	92	11 ·6	168	18 ·4	320	28 ·8	512	44 ·8	992	2,240
	50	20	29	48	84	11 ·6	160	17 ·6	296	28 ·8	464	48 ·0	896	2,752
	Mean	20	32 ·6	55 ·6	93 ·0	12 ·3	170 ·4	19 ·5	290 ·4	27 ·8	483 ·2	45 ·8	976 ·0	2,268 ·8

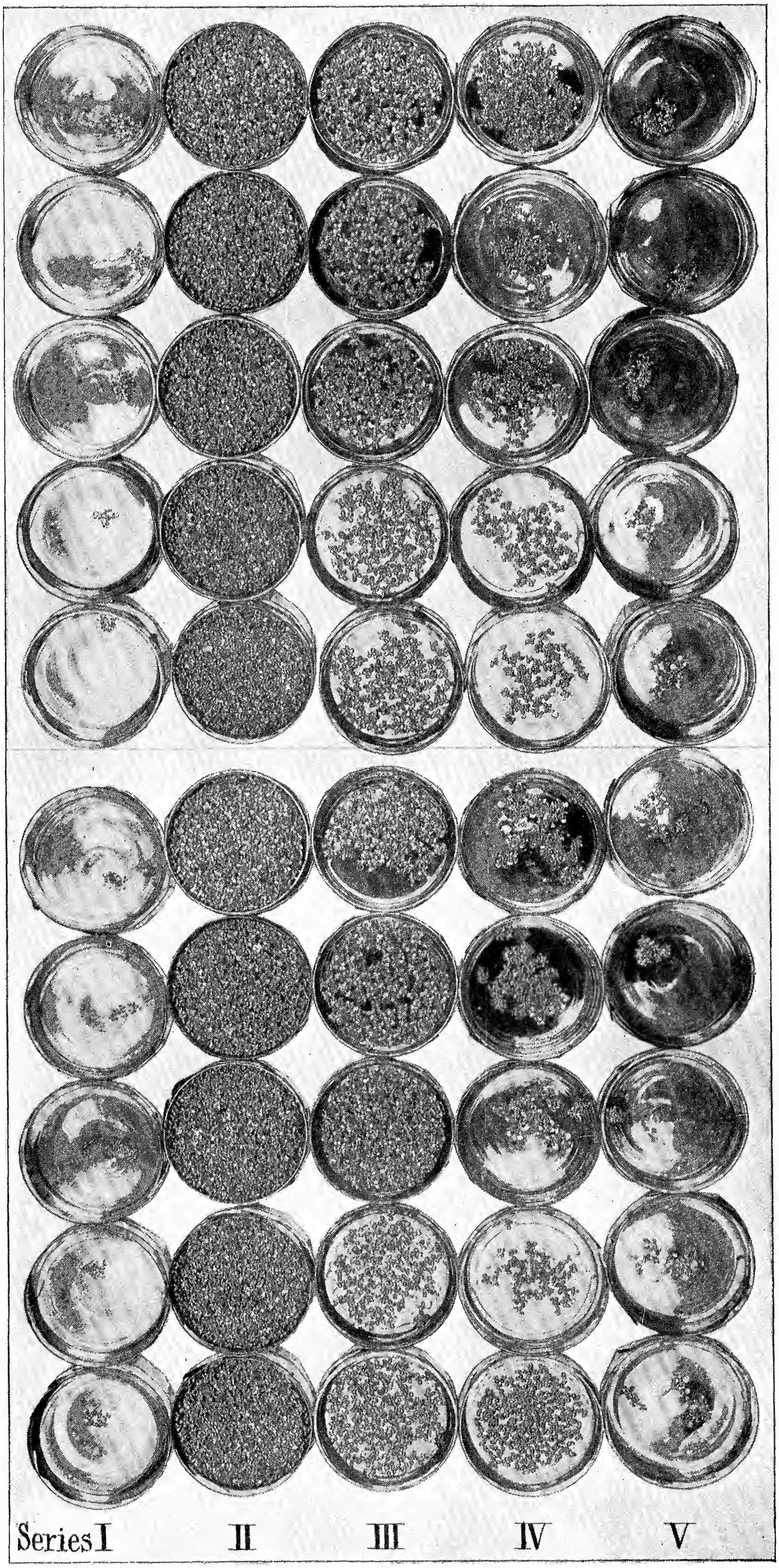
The total number of plants each week, and their dry weight for the 3rd and succeeding weeks, are shown in the Table on pp. 486–7, the numbers representing, after the 2nd week, not those which were actually in the dishes, but the numbers which would have resulted from the multiplication of the original 20 had the dishes used been sufficiently large to obviate the need for halving and quartering. The dry weights also correspond to the weights of the complete sets whose numbers are given, and not to the fractions of the whole sets which were weighed each week. By the end of the 6th week, the labour involved in counting, halving, and estimating the dry weight of the plants in the 50 dishes had become so great that it was impossible to continue the whole of the work further. The experiment was carried on, however, for another two weeks, with the object of recording only the numbers of the plants in the various series.

It is evident from the above figures that all the additions to Detmer's solution have a beneficial effect on the growth of the *Lemna minor* in water culture, and that there is a progressive beneficial effect both in number and weight as the amount of added substances is increased. The most striking results are shown in Series II with the complete extract of bacterised peat, which in six weeks increased the numbers by 20 times (6723 : 326), and the weights by 62 times (1103 : 17·6), those in Detmer's solution. As the additions made consisted chiefly of organic substances, the conclusion appears to be justified that it is the presence of organic matter which is responsible for the results obtained, for it is difficult to understand how such increases could have been brought about by the addition of inorganic nutrients only, since the maximum proportions of nitrogen, phosphorus and potash added in Series II amounted to only 4·8, 1·8 and 0·04 per cent. respectively of those already present in Detmer's solution.

The numbers obtained during the first six weeks are shown graphically below, together with a diagrammatic representation of the maximum percentage addition of nitrogen, phosphorus and potash to the quantities present in Detmer's solution.

A photograph of the whole set of 50 dishes, taken during the 6th week, is shown on Plate 21.

A comparison between the relative numbers and weights of plants in all the series is more readily obtained by putting the average number and weight of plants in Series I at 100 for each week, and ascertaining the corresponding figures, which show the percentage of increase, for the other series. These are shown in the Table below.


Series I
II
III
IV
V

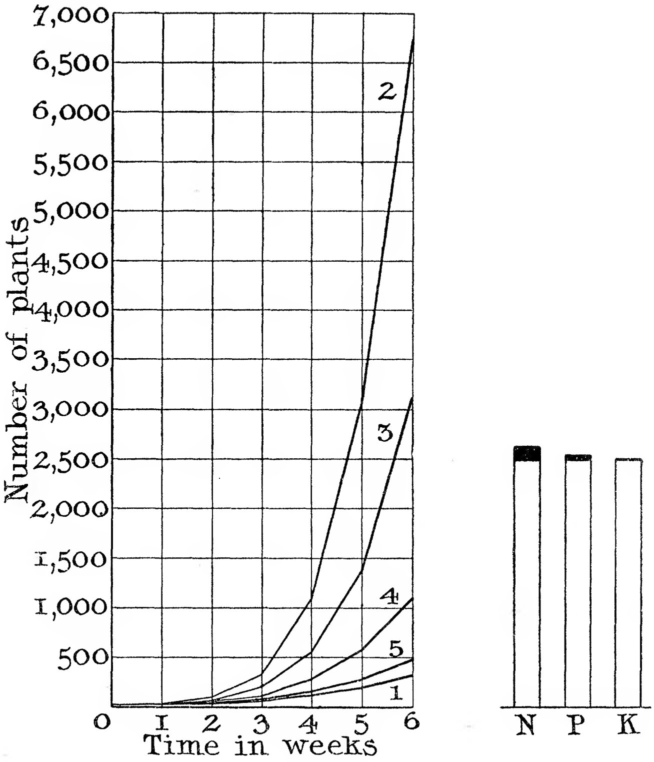


Fig. 1

1. Detmer's solution.
2. „ „ +water extract.
3. „ „ +water extract (free from humic acid).
4. „ „ +alcoholic extract.
5. „ „ +phosphotungstic fraction.

(The black portions at the top of each column represent the maximum percentage addition (Series II) of N (4·8 per cent.), P (1·8 per cent.), and K (0·04 per cent.), to the quantities already present in Detmer's solution.)

Table IV.

Series.	At beginning of experiment.	1st week.	2nd week.	3rd week.		4th week.		5th week.		6th week.		7th week.	8th week.
		No.	No.	No.	Wt.	No.	Wt.	No.	Wt.	No.	Wt.	No.	No.
I	100	100	100	100	100	100	100	100	100	100	100	100	100
II	100	123	195	426	536	814	1,519	1,451	4,100	2,059	6,267	3,590	6,587
III	100	117	145	262	288	415	690	660	1,711	957	2,934	1,774	3,684
IV	100	109	114	159	166	207	255	256	492	337	736	580	1,232
V	100	107	107	121	122	126	164	137	223	148	260	177	215

A graphical representation of these figures is shown in the following diagram:

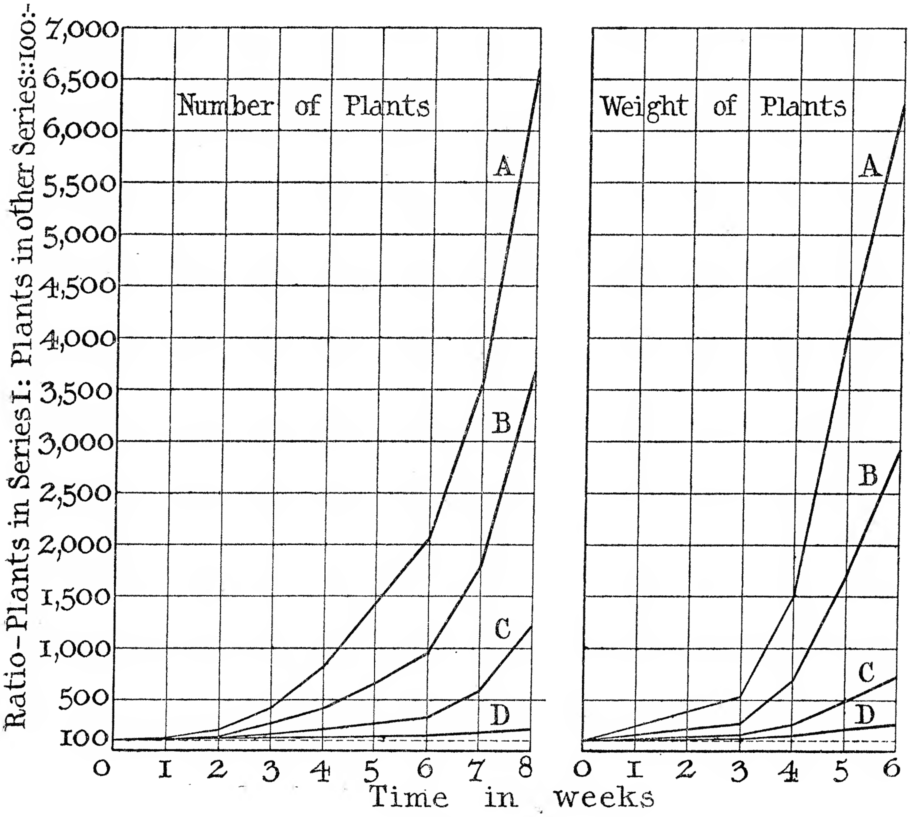


Fig. 2.

A. Series II: Detmer + water extract of bacterised peat.
B. „ III: „ + water extract free from humic acid.
C. „ IV: „ + alcoholic extract.
D. „ V: „ + phosphotungstic fraction.

The marked superiority in size, as indicated by weight, of the plants supplied with auximones is shown in the Table below. The mean weight of five sets, each consisting of 100 plants, at the beginning of the experiment was 12 mg., and the corresponding weights of 100 plants in every series was calculated from the third week onwards, from the average weights and average numbers obtained from all ten dishes in each series.

Table V.

Series.	At beginning of experiment.	3rd week.	4th week.	5th week.	6th week.
	mgrm.	mgrm.	mgrm.	mgrm.	mgrm.
I	12·0	13·0	8·8	5·9	5·4
II	12·0	16·4	16·5	16·7	16·4
III	12·0	14·3	14·7	15·3	16·5
IV	12·0	13·6	10·8	11·3	11·8
V	12·0	13·1	11·5	9·6	9·0

These figures are graphically represented in the diagram below.

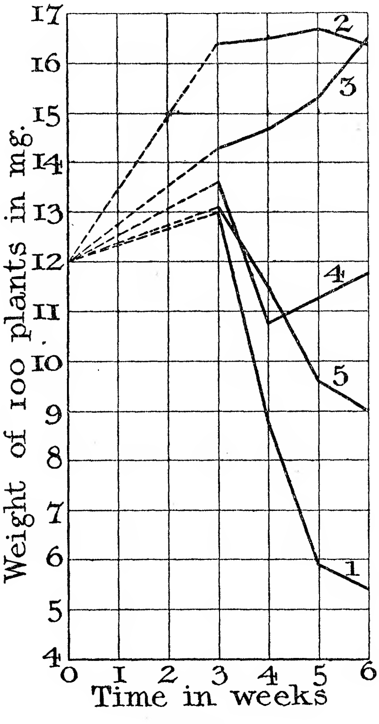


Fig. 3.

1. Series I: Detmer's solution.
2. „ II: „ „ + complete water extract.
3. „ III: „ „ + water extract free from humic acid.
4. „ IV: „ „ + alcoholic extract.
5. „ V: „ „ + phosphotungstic fraction.

A measure of the comparative rapidity with which the plants in each series doubled their number and their weight is obtained by putting the number of times which the plants of Series I doubled themselves in a given time at unity, and calculating the corresponding figures for each of the other series. The figures thus obtained are given below.

Table VI.

Series.	Comparative rate of doubling in a given time.		Units of time required to double.	
	No.	Wt.	No.	Wt.
I	1·0	1·0	100	100
II	2·05	3·09	48	32
III	1·91	2·71	52	37
IV	1·63	2·01	61	50
V	1·24	1·48	80	67

Very little difference was noticed in the appearance of the plants in the various dishes for the first week, but during the second week the effect of the auximones became apparent, especially in Series II and III, although it was not until the third week that the effect in Series IV and V became well marked. The plants in Detmer's solution gradually assumed a starved appearance which became very marked as the experiment progressed. They gradually lost their green colour, and after the third week their decrease in size was very noticeable. The auximone effect was shown by increased rapidity of multiplication of the plants, larger size, stronger root development and greener colour; that is, essentially, by a more vigorous and healthy growth.

It is clearly evident from the results obtained in the various series that successive fractionation of the extracts obtained from bacterised peat resulted in a diminution of the effective growth-promoting substances present. The best results were shown in Series II, where the whole of the water-soluble substances were supplied, while in Series III, where soluble humate was eliminated from this water extract, growth was not so good. This may have been due partly to the absence of soluble humate as such and partly to the removal of certain organic substances adsorbed in the precipitated calcium humate. Again, in Series IV, the elimination of all except the alcohol-soluble substances resulted in a much less marked, though still very appreciable, effect; and this was still further emphasised in Series V, where the decomposed phosphotungstic-acid fraction was employed. These facts are brought out in the following diagram, in which the five verticals are drawn to represent the five series shown in Table III. The total mean number and weight of the plants in Series I at the end of six weeks are taken to represent unity, and this is marked off on the first ordinate, the corresponding numbers for the other series being calculated, both for weight and number, and marked off on the successive ordinates. Upon joining up these points, two steadily rising curves are obtained for the numbers and weights, corresponding to an ascending order of complexity in the fractions supplied.

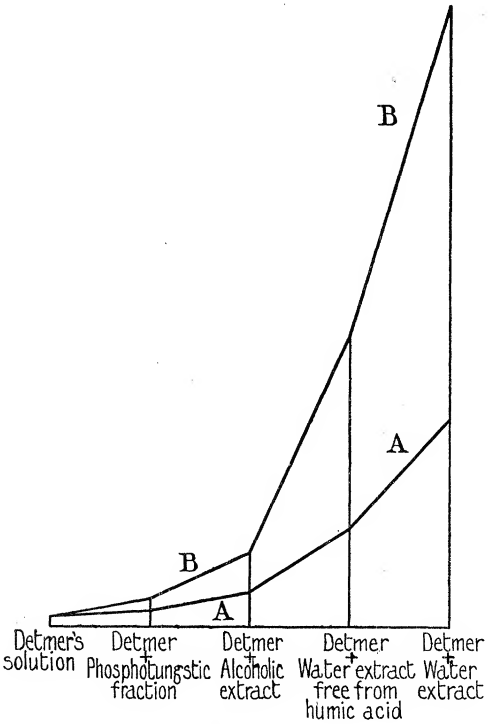


FIG. 4.

It is of interest here to compare the effect of auximones in the 1915 experiments with that shown above. The only comparable figures are those obtained with the phosphotungstic fraction for the first seven weeks of each experiment, since no figures are available for the 8th week. The figures for this period of the experiments shown in Table III give a percentage increase in number over the control plants of 77·4, while the preceding year's figures show an increase of 64·5 per cent. for the corresponding period. Allowing for the variation in conditions of weather and environment it is thus evident that there is a close agreement between the results obtained during the two seasons.

Experiments with Conductivity Water.

In view of the well-known fact that ordinary distilled water such as is commonly used in the laboratory contains traces of toxic substances, it was thought possible that some of the beneficial effects of the added organic matter in the previous experiments might be due to a neutralisation of the toxicity of the distilled water used.

Owing to the limited supply of glass-distilled or "conductivity" water available, it was impossible to duplicate the whole of the series, but for the purpose of testing how far the diminution in size and vigour of the plants grown in ordinary distilled water, in Series I of the previous experiment, was due to any toxicity of this water, an additional series of ten dishes, precisely similar to Series I, except for the fact that conductivity water was used instead of the ordinary distilled water, was prepared, and this ran concurrently with the rest of the series. This set was numbered from 51 to 60, and was known as Series VI.

The fact that these plants multiplied more rapidly and retained their green colour longer than those in Series I indicated that this water was less toxic than the ordinary distilled water. By the third week, however, when the first halving of the whole set was made, the diminution in size of these plants was very marked. It was therefore decided that, instead of estimating the dry weight of one-half of the plants in each dish in this series at this stage, these plants should be used for testing the effect in conductivity water of certain auximones. As only sufficient conductivity water was available for another ten dishes, and it was desirable that two of the auximone fractions should be tested, two small series, each consisting of five dishes only, were employed. This reduction in the number of the dishes in the new sets appeared to be justified at this stage by the uniformity in the number of plants which prevailed throughout the whole of the dishes in any one series of the above experiment.

The halves of dishes 51 to 55 respectively were transferred to dishes numbered 61 to 65, containing a solution similar to that used in Series III, but made up in conductivity water. This set formed Series VII. Similarly, the halves of dishes numbered 56 to 60 formed another series containing an alcoholic extract of bacterised peat as in Series IV. Unfortunately, the plants in this series very soon became attacked by a fungal disease, and were eventually discarded. At the 7th week of the experiment this series was replaced by another consisting of the halves of the contents of dishes 56 to 60 at this date. This set was numbered from 66 to 70 and formed Series VIII, containing a culture solution similar to that in Series II, made up in conductivity water. The peat extract used here, however, was reduced to half strength, the extract from 1 grm. being used in every 1000 c.c., instead of 500 c.c. as in Series II. This represented a concentration of 184 parts per million of organic matter. These series in conductivity water

Table VII.

Numbers.	Dish 51.	Dish 52.	Dish 53.	Dish 54.	Dish 55.	Dish 56.	Dish 57.	Dish 58.	Dish 59.	Dish 60.	Mean.
At beginning ...	20	20	20	20	20	20	20	20	20	20	20
1st week	22	24	20	21	20	21	20	22	21	22	21·3
2nd ,, ...	52	51	50	44	46	47	53	53	55	54	50·5
3rd ,, ...	118	106	118	110	114	102	114	100	126	108	111·6
4th ,, ...	232	212	236	204	264	208	240	212	252	220	228·0
5th ,, ...	376	368	472	360	432	352	464	336	464	352	397·6
6th ,, ...	704	672	784	544	736	608	784	528	816	656	683·2
7th ,, ...	1,600	1,312	2,016	928	1,728	1,568	1,888	1,088	1,888	1,216	1,523·2
8th ,, ...	3,008	1,728	2,624	1,344	2,240	2,816	3,392	1,408	3,328	2,048	2,393·6
9th ,, ...	4,864	2,816	3,712	2,816	3,712	3,200	4,480	3,072	4,992	3,840	3,750·4
10th ,, ...	6,400	6,656	6,656	5,632	4,608	5,632	6,656	4,864	7,680	5,632	6,041·6
11th ,, ...	13,824	12,288	12,288	12,800	8,704	11,776	14,336	9,728	14,336	10,752	12,083·2
12th ,, ...	23,552	20,480	27,648	19,456	14,336	23,552	24,576	20,480	25,600	16,384	21,606·4
13th ,, ...	49,152	40,960	55,296	47,104	30,720	47,104	47,104	45,056	53,248	34,816	45,056·0
Weight—	mgrm.	mgrm.	mgrm.	mgrm.	mgrm.	mgrm.	mgrm.	mgrm.	mgrm.	mgrm.	mgrm.
At beginning ...	2·4	2·4	2·4	2·4	2·4	2·4	2·4	2·4	2·4	2·4	2·4
4th week	16·8	13·6	20·8	23·2	24·8	16·0	18·4	15·2	28·0	20·8	19·8
5th ,, ...	36·8	32·0	43·2	30·4	41·6	30·4	33·6	35·2	38·4	41·6	36·3
6th ,, ...	60·8	57·6	70·4	54·4	64·0	60·8	70·4	60·8	76·8	48·0	62·4
7th ,, ...	115·2	121·6	179·2	76·8	140·8	—	—	—	—	—	126·7
8th ,, ...	204·8	208·8	211·2	83·2	166·4	224·0	200·8	121·6	211·2	134·4	176·6
9th ,, ...	258·4	307·2	307·2	179·2	204·8	279·2	204·8	153·6	307·2	179·2	238·1
10th ,, ...	409·6	358·4	409·6	460·8	256·0	460·8	409·6	409·6	665·6	256·0	409·6
11th ,, ...	1,126·4	921·6	614·4	819·2	512·0	819·2	1,024·0	512·0	921·6	614·4	788·5
12th ,, ...	1,228·8	1,843·2	1,638·4	1,433·6	614·4	1,228·8	1,024·0	1,843·2	1,843·2	614·4	1,331·2
13th ,, ...	3,686·4	2,867·2	2,048·0	2,048·0	2,867·2	3,686·4	1,228·8	2,457·6	2,457·6	2,048·0	2,539·5

ran until the 13th week from the commencement of Series VI, and the figures which would then have been obtained from each complete set of 20 plants in Series VI are shown in the Table on the preceding page.

The corresponding numbers for the five dishes in Series VII can be given for only 11 weeks instead of 13, since this was not started until the 3rd week; and it should be noted that the numbers in the dishes of this series at the beginning of the experiment are not uniformly 20 as in the previous series, but show a variation, for they represent the halves of the contents of dishes 51 to 55 respectively of Series VI in its 3rd week.

Table VIII.

	Dish 61.	Dish 62.	Dish 63.	Dish 64.	Dish 65.	Mean.
Number of plants—						
3rd week (at beginning)	59	53	59	55	57	56 ·6
4th week …………	128	122	138	128	130	129 ·2
5th „ …………	288	288	292	284	308	292 ·0
6th „ …………	592	592	608	616	664	614 ·4
7th „ …………	2,144	2,192	1,872	2,192	2,112	2,102 ·4
8th „ …………	7,872	8,640	5,920	9,024	8,064	7,904 ·0
9th „ …………	26,240	20,864	22,272	24,960	24,320	23,731 ·2
10th „ …………	51,456	56,320	50,176	52,736	48,640	51,865 ·6
11th „ …………	110,080	122,880	114,176	110,080	107,008	112,844 ·8
12th „ …………	201,728	222,208	228,352	215,040	211,968	215,859 ·2
13th „ …………	460,800	487,424	538,624	460,800	454,656	480,460 ·8
Weight of plants—	mgrm.	mgrm.	mgrm.	mgrm.	mgrm.	mgrm.
4th week …………	10 ·8	11 ·6	14 ·0	12 ·4	12 ·4	12 ·2
5th „ …………	38 ·4	36 ·0	36 ·8	33 ·6	34 ·4	35 ·8
6th „ …………	81 ·6	86 ·4	96 ·0	84 ·8	84 ·8	86 ·7
7th „ …………	297 ·6	313 ·6	265 ·6	288 ·0	313 ·6	295 ·7
8th „ …………	1,004 ·8	1,145 ·6	851 ·2	1,017 ·6	1,011 ·2	1,006 ·1
9th „ …………	2,982 ·4	2,867 ·2	2,944 ·0	2,713 ·6	2,841 ·6	2,869 ·8
10th „ …………	5,734 ·4	7,270 ·4	6,451 ·2	7,065 ·6	6,451 ·2	6,594 ·6
11th „ …………	14,848 ·0	16,998 ·4	15,667 ·2	17,100 ·8	14,745 ·6	15,872 ·0
12th „ …………	28,876 ·8	36,249 ·6	36,454 ·4	33,996 ·8	33,587 ·2	33,832 ·9
13th „ …………	68,403 ·2	81,100 ·8	87,654 ·4	70,041 ·6	78,233 ·6	77,086 ·7

Similarly in the case of Series VIII the figures, shown in the Table below, can be given for seven weeks only, since this series was not commenced until the 7th week of the whole experiment (Table IX).

A correct comparison of the rates of growth in these three series cannot be obtained by comparing the figures in the columns for corresponding weeks, since the three series were all started at different dates. The comparison can only be made by putting the number of plants in Series VI at 100 for each week, and calculating the corresponding numbers for Series VII and VIII. It must be noted, however, that Series VII was started at the 3rd week of Series VI by taking one-half of the contents of the dishes of

Table IX.

	Dish 66.	Dish 67.	Dish 68.	Dish 69.	Dish 70.	Mean.
Number of plants—						
7th week (at beginning)	49	59	34	59	38	47 ·8
8th week	138	204	106	180	90	143 ·6
9th "	524	680	444	648	480	555 ·2
10th "	1,056	1,344	976	1,360	840	1,115 ·2
11th "	2,944	3,232	2,208	3,792	2,128	2,860 ·8
12th "	6,272	7,360	4,320	5,920	4,160	5,606 ·4
13th "	16,576	19,008	12,800	22,656	10,944	16,396 ·8
Weight of plants—	mgrm.	mgrm.	mgrm.	mgrm.	mgrm.	mgrm.
8th week	16 ·0	19 ·6	9 ·6	16 ·0	11 ·2	14 ·5
9th "	56 ·0	66 ·4	52 ·0	67 ·2	45 ·6	57 ·4
10th "	123 ·2	142 ·4	116 ·8	148 ·8	83 ·2	112 ·9
11th "	355 ·2	412 ·8	300 ·8	489 ·6	272 ·0	366 ·1
12th "	1,004 ·8	1,126 ·4	736 ·0	953 ·6	652 ·8	894 ·7
13th "	2,624 ·0	2,764 ·8	2,086 ·4	3,648 ·0	1,817 ·6	2,588 ·2

Series VI to start the new series. The numbers given in the Table for Series VI, however, represent not the halves which were left and which would correspond with the new series, but the complete sets which would have been obtained had the contents of the dishes not been halved. It is evident, therefore, that the numbers for Series VII from the 3rd week onwards correspond to one-half of the numbers given for Series VI for the same period, and that, in order to obtain a correct comparison, one-half of the mean numbers for Series VI must be put at 100, and the corresponding numbers for Series VII then calculated. Similarly, Series VIII was started at the 7th week of Series VI, at the fifth time of halving these dishes. One thirty-second part of the complete sets for Series VI was therefore used to start Series VIII, and the numbers for the latter series from the 7th week onwards correspond to 1/32 of the numbers given for Series VI. In order therefore to correctly compare the rate of growth in these two series, the mean numbers for Series VI must be divided by 32, and the resulting figures put at 100, to obtain the corresponding numbers for Series VIII. A comparison thus obtained for both numbers and weights is given in Table X on p. 498.

The average weekly weight of 100 plants in each of the three series is shown in Table XI on p. 498.

The drop in the average weight of the plants in Series VII from the 7th to the 9th week is probably explained by the excessive multiplication of the plants during a fortnight of extremely hot weather An abnormally large number of small new plants were thus included in the weekly countings, and this resulted in a smaller average weight.

Table X.

Series.		3rd week.	4th week.	5th week.	6th week.	7th week.	8th week.	9th week.	10th week.	11th week.	12th week.	13th week.
VI. Detmer	No.	100	100	100	100	100	100	100	100	100	100	100
	Wt.	100	100	100	100	100	100	100	100	100	100	100
VII. Detmer + water extract free from humic acid	No.	100	113	147	179	276	660	1265	1716	1867	1998	2133
	Wt.	100	123	198	278	467	1139	2411	3220	4026	5083	6071
VIII. Detmer + water extract	No.	—	—	—	—	100	192	466	591	759	852	1164
	Wt.	—	—	—	—	100	263	771	882	1486	2150	3261

Table XI.

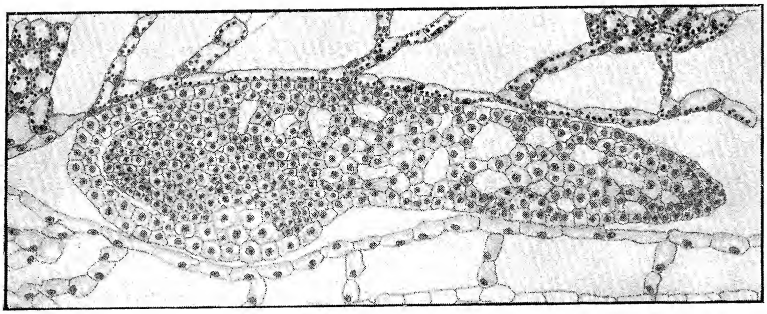
	Weight of 100 plants.										
	At beginning.	4th week.	5th week.	6th week.	7th week.	8th week.	9th week.	10th week.	11th week.	12th week.	13th week.
	mgrm.	mgrm.	mgrm.	mgrm.	mgrm.	mgrm.	mgrm.	mgrm.	mgrm.	mgrm.	mgrm.
Series VI: Detmer's solution	12·0	8·7	9·1	9·1	8·3	7·4	6·3	6·8	6·5	6·1	5·6
Series VII: Detmer + water extract free from humic acid	—	9·5	12·3	14·1	14·1	12·7	12·1	12·7	14·0	15·7	16·0
Series VIII: Detmer + water extract	—	—	—	—	—	10·1	10·3	11·0	12·8	15·9	15·8

It is evident from these figures that, whilst the toxic substances in the metal-distilled water employed in the previous experiments has had a certain injurious effect upon the growth of the plants, yet the use of pure non-toxic water with mineral salts will not suffice for normal and healthy growth for any length of time. Although the plants in conductivity water multiplied more rapidly, retained their healthy appearance longer, and decreased in weight less rapidly than those in ordinary distilled water, yet the final result in each case was the same.

These figures are graphically represented in the diagram, fig. 5.

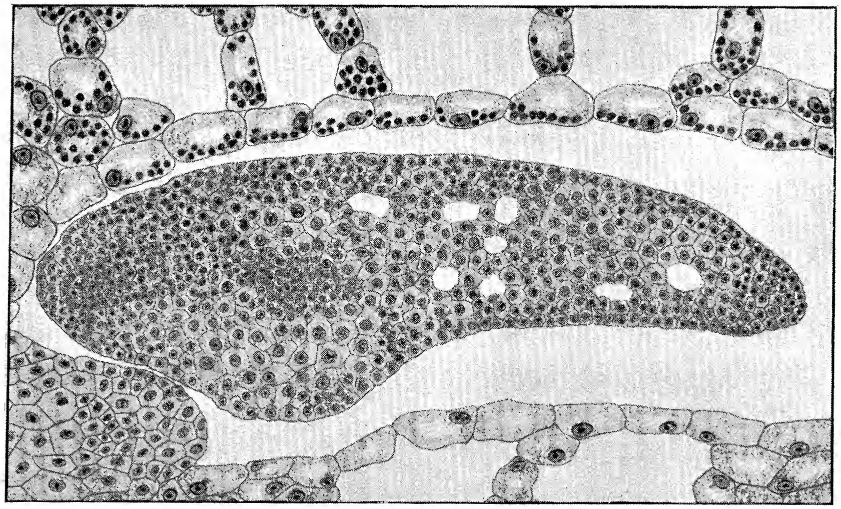
In view of the striking differences in general appearance between the control and auximone plants in both ordinary distilled water and conductivity water, an investigation was made of the internal structure of representative plants from each set at the conclusion of the experiment. The plants were fixed, microtomed and stained in the usual manner. Microscopic examination then showed that in all the plants examined receiving auximones, the tissues were more dense, and the proportion of air spaces to

Section through young frond. Series VI. × 235.



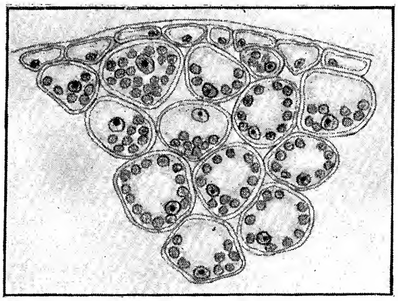
– Auximones.

Section through young frond. Series VIII. × 235.



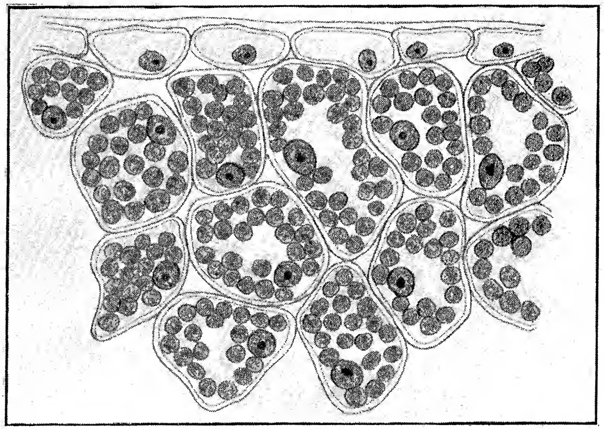
+ Auximones.

Typical cells from old frond. Series VI. × 610.



– Auximones.

Typical cells from old frond. Series VIII. × 610.



+ Auximones.

cellular tissue was much less than in the control plants. The difference was also very evident in the individual cells. In the auximone plants they were larger and more densely filled with protoplasm, containing larger nuclei and more numerous and larger chloroplastids than in the control plants. This difference was especially noticeable at the apex of young, newly-formed plants.

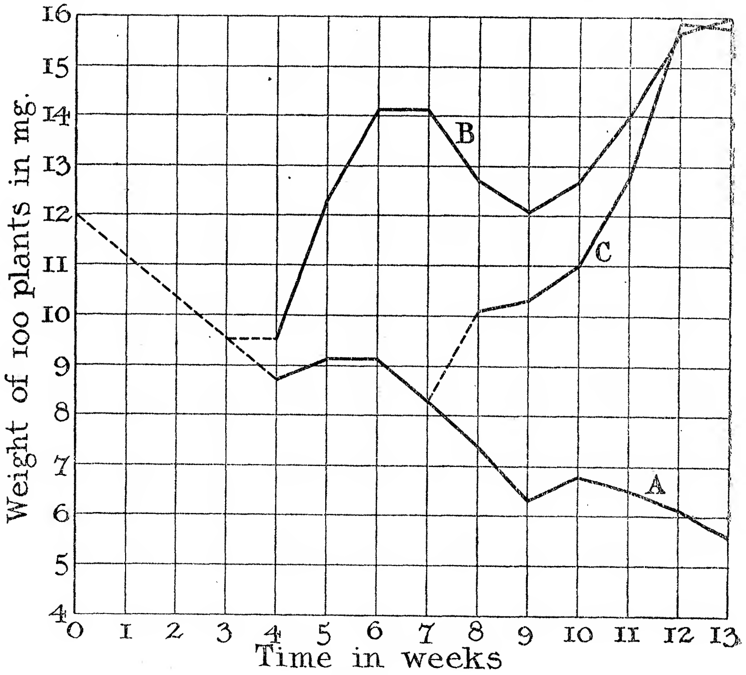


FIG. 5.

A. Series VI : Detmer's solution in conductivity water.
B. „ VII : „ „ + water extract free from humic acid.
C. „ VIII : „ „ + complete water extract.

These various points are evident from the diagrams on Plate 22, drawn with the camera lucida, of representative plants grown in Series VI and VIII in conductivity water, the two of each set being of the same magnification.

Interchange of Culture Solutions.

When it became evident from the previous experiments that a period of three weeks generally elapsed before the effect of added auximones became very marked in plants which had been taken from their normal habitat, a further experiment was commenced at King's College to ascertain how soon plants grown in water culture with and without auximones would respond to a reversal of these conditions.

Two series of five dishes each were prepared containing solutions similar to those in Series I and II above. The dishes were numbered from 1 to 10, Nos. 1 to 5 forming Series I with Detmer's solution, and Nos. 6 to 10 Series II with Detmer's solution plus complete water extract. Twenty uniform plants of *Lemna minor* were placed in each dish, and the sets treated precisely as in the preceding experiments for five weeks. Twenty average plants were then counted out from each dish and the culture solutions in each series were transposed; that is, the five sets each of 20 plants previously growing in Detmer's solution were now in Detmer's solution plus auximones (Series I), while those hitherto supplied with auximones were now growing in Detmer's solution alone (Series II). These plants were allowed to grow on for another nine weeks, receiving the same treatment as to changing and counting as before. At this stage 20 plants from each series were again transposed, that is, were transferred to their original solutions, and these were counted for a further six weeks. The numbers in the five dishes of each series approximated very closely to the mean for the series throughout, so only the average numbers are given in the Table below:—

Table XII.

Period I.			Period II.			Period III.		
	Series I. − Auximone.	Series II. + Auximone.		Series I. + Auximone.	Series II. − Auximone.		Series I. − Auximone.	Series II. + Auximone.
At beginning	20	20	5th week (at beginning)	20	20	14th week (at beginning)	20	20
1st week	30	46	6th week ...	49	58	15th week ...	97	89
2nd ,, ...	64	104	7th ,, ...	102	113	16th ,, ...	402	458
3rd ,, ...	122	235	8th ,, ...	201	201	17th ,, ...	722	957
4th ,, ...	192	630	9th ,, ...	688	490	18th ,, ...	1,704	2,689
5th ,, ...	426	1,528	10th ,, ...	2,348	1,212	19th ,, ...	3,288	5,606
			11th ,, ...	7,552	2,956	20th ,, ...	7,648	14,720
			12th ,, ...	23,910	6,707			
			13th ,, ...	108,953	23,142			
			14th ,, ...	372,531	73,318			

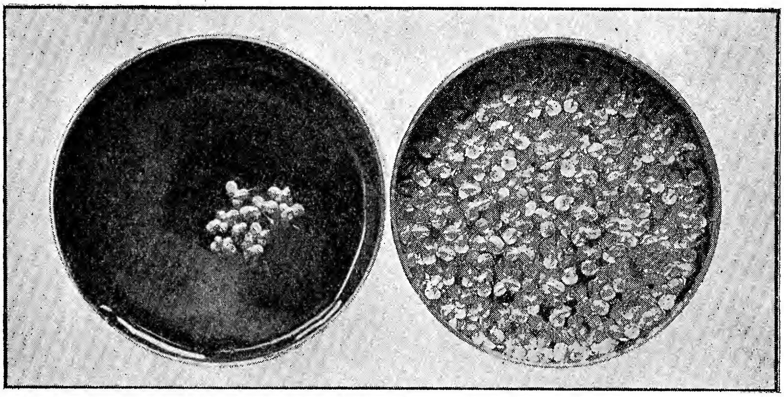
It is very evident from the above figures that whenever auximones are present in the culture solutions the plants multiply much more rapidly than in mineral nutrients alone. This is brought out by a comparison of the final figures for each period.

Period I.	Detmer − auximones	426	Detmer + auximones	1,528
Period II.	Detmer + auximones	372,531	Detmer − auximones	73,318
Period III.	Detmer − auximones	7,648	Detmer + auximones	14,720

This comparison is also well shown by the following photographs of one typical dish from each series taken during Periods I and II respectively:—

Period I.

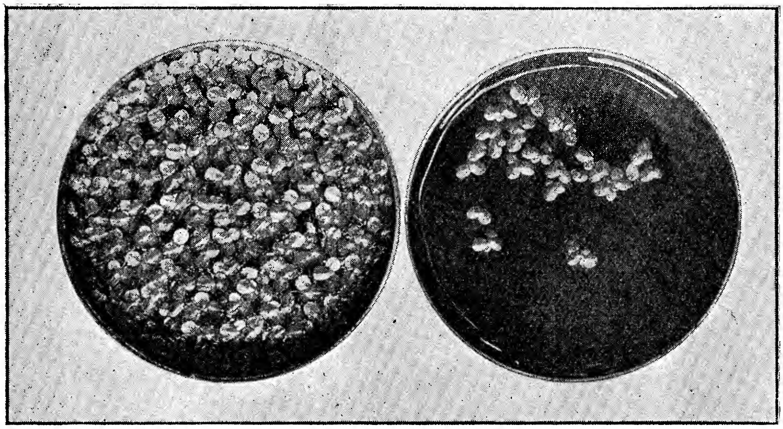
Series I. Series II.



− Auximones. + Auximones.

Period II.

Series I. Series II.



+ Auximones. − Auximones.

Fig. 6.

A comparison of the rate of growth in the different culture solutions is best obtained as before by putting the average number of plants in Series I at 100 for each week, and estimating the corresponding numbers for Series II. The figures thus obtained are:—

Table XIII.

Series I.	Series II (1st period).		Series II (2nd period).		Series II (3rd period).	
100 throughout ...	At beginning	100	5th week .... (at beginning)	100	14th week .... (at beginning)	100
	1st week......	152	6th week	118	15th week	92
	2nd ,, ...	162	7th ,, ...	111	16th ,, ...	114
	3rd ,, ...	193	8th ,, ...	100	17th ,, ...	132
	4th ,, ...	326	9th ,, ...	71	18th ,, ...	158
	5th ,, ...	359	10th ,, ...	52	19th ,, ...	170
			11th ,, ...	39	20th ,, ...	192
			12th ,, ...	28		
			13th ,, ...	21		
			14th ,, ...	20		

These figures are represented graphically in the diagram below:—

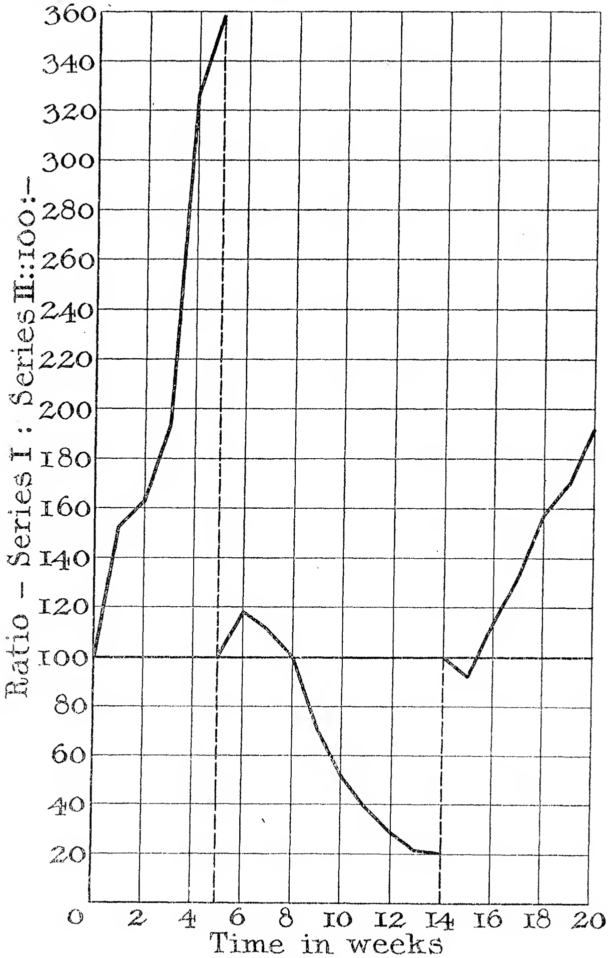


FIG. 7.

From this diagram it is seen that plants which have been growing for some time in either Detmer's solution or Detmer's solution plus auximones do not respond immediately to the addition or removal of these substances to or from the culture solution. There is about a week—a "reaction time"—during which the previous tendency to decrease or increase is manifest before the new factor becomes effective.

An important point, which was noticed throughout the experiments, was the fact that the greatest variation in the numbers and weights of the plants in the various dishes each week appears in the control sets. Evidently, where the plants are lacking auximones, there is a lack of uniformity between the rate of multiplication and the increase in weight of the plants. In some weeks growth-energy appears to be expended in multiplication, with little corresponding increase in weight; in other weeks increase in weight appears to be attained at the expense of numbers. For example, in Table VI, Series VI, Dish 58 in conductivity water shows a doubling of number from the 10th to the 11th week, but the weight has only increased by about 25 per cent. during the same period. From the 11th to the 12th week the same dish shows a further increase of rather more than 100 per cent. in the number, but this time it is accompanied by an increase of more than 250 per cent. on the weight. Similarly, in Table II, Series II, Dish 4 in ordinary distilled water shows an increase in number of practically 50 per cent. from the 4th to the 5th week, but the weight has scarcely increased at all, while from the 5th to the 6th week, an increase in number of about 60 per cent. is accompanied by an increase in weight of about 50 per cent.

In the series containing auximones there is a much greater conformity between the rate of multiplication and increase in weight.

Conclusions.

The foregoing experiments appear to justify the conclusions (1) that bacterised peat contains certain organic substances which, when supplied even in small quantities to *Lemna* plants growing in complete mineral culture solutions, have a remarkable effect upon their growth; (2) that in these plants normal growth and multiplication cannot be sustained for any length of time in the absence of these organic growth-promoting substances or auximones; (3) that these substances are essential for the effective utilisation and assimilation of the mineral nutrients supplied to these plants.

It is highly probable that these auximones are organic decomposition products, for bacterised peat is simply organic matter, already partially decomposed by anaërobic action, which has been further decomposed by the agency of aërobic bacteria under suitable conditions. Hence the active growth-promoting substances obtained in solution from this must be products of decomposition of organic matter. Further experiments, not yet published, have shown that these substances are also obtainable, in relatively small amounts, from well-rotted stable manure and thoroughly decomposed leaf-mould. It is to be expected, therefore, that small quantities would be formed among the products of organic decay in the water of a pond, and it is interesting to note, in this connection, that in nature *Lemna* plants flourish best in stagnant water, rich in organic decomposition products.

Until more is known as to the nature of these products, it is impossible to state definitely how they function. Some of them may be absorbed and utilised directly as plant nutrients. Schreiner and Skinner* have shown that such nitrogenous decomposition products as creatinine, histidine and arginine can replace nitrates in a culture solution, and that, even when nitrates are present, these substances are absorbed by the plants. They say that "these compounds are absorbed as such, and utilised directly for building up the proteins and other complex nitrogenous constituents of vegetable material." They also suggest that the energy usually employed in a plant in effecting the chemical transformation of inorganic nitrogen into an organic form can be expended otherwise when these substances are supplied to the plant, and thus "plant efficiency is increased and growth augmented when the plant obtains compounds which will serve directly as tissue builders."

On the other hand, some may have a similar effect to that of the accessory food bodies or growth vitamines concerned in animal growth. The phosphotungstic fraction of bacterised peat was obtained by precisely the same method as that used for extracting animal growth vitamines, and it is difficult to understand how the addition of such a small amount of organic matter as 13 parts per million from this material to a culture solution already containing 5500 parts per million of mineral nutrient salts could produce the results obtained if it represented only a further addition of plant nutrient. The possibility that such accessory food bodies are present in bacterised peat is emphasised by the fact that the water extract free from humic acid, the alcoholic extract, and the phosphotungstic fraction, have all been found to give a positive reaction with the Folin-Macullum phosphotungstic acid reagent. This positive reaction, which is not given by the corresponding fractions of raw peat, is considered by Williams and

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* Schreiner and Skinner, U.S. Dept. Agric., Bureau of Soils, Bull. 87, 1912.

Seidell* to indicate, with certain reservations, the presence of animal growth vitamines.

It is possible that both organic plant nutrients and accessory food substances were present in the complex water extract of bacterised peat supplied in the above experiments, and that these had a co-operative effect, for, even if one assumes that the phosphotungstic fraction contained only accessory food substances, it is seen that the more complex water extract produces the best growth results.

In view of the generally accepted botanical theory that green plants can build up complex protein compounds from mineral salts, and mineral salts only, the above results are difficult to explain. If this theory be correct, then *Lemna minor* must either have lost this power, or it is an exception to the general rule, since it is evident that, for healthy growth, this plant must be supplied with small quantities of certain organic substances in addition to mineral nutrients. The beneficial effect of organic manures in cultural operations is well known. Recently Livingston† has shown that manure extract and the expressed juice of red-clover leaves have a remarkable effect in increasing the growth of wheat seedlings in culture solutions consisting of soil extracts and mineral nutrients. He demonstrates that it is the organic matter of the extracts which is beneficial, but he attributes the benefit to "some correcting influence which it brought to bear upon the toxic bodies of the soil extract and those which appear to be produced by the seedlings" rather than to a direct action upon the nutrition of the plants.

The remarkable effect of the addition of organic substances in the experiments on *Lemna minor* recorded above, as manifested not only by the enormous increase in number and weight of the plants, but also by the increased vigour of the individual cells, as shown by the denser protoplasm, larger nuclei and more numerous chloroplastids, indicates that the organic substances play some definite and essential part in the general metabolic activities of the plant.

To what extent certain organic substances may be necessary for the growth of green plants in general, further experiments alone can decide. The well-known fact that the seedlings of land plants can be grown to maturity in culture solutions of mineral salts is not a fatal objection to the suggestion that all green plants may require traces of certain organic substances for their optimum development, since it has been shown that nitrogenous organic growth-promoting substances are produced during the germination of seeds. Brown* has shown that during the germination of barley seeds, certain soluble nitrogenous substances, which are essential for the early stages of development of the young plant, are formed in the endosperm and absorbed by the embryo. The writer also has found that a water extract of germinated seeds has a similar effect on *Lemna* plants growing in culture solution to that of bacterised peat, while no growth-promoting substances could be obtained from dry seeds. It is probable, therefore, that the seedlings used in the ordinary water-culture experiments may already contain the necessary minimal quantities of organic substances requisite for ordinary growth, but that a further supply of these substances is essential for optimum growth and development. It is therefore not unreasonable to assume that organic growth-promoting substances are as essential for plant as for animal nutrition, since the difference in metabolism between plants and animals is one of degree only; and that just as the plant is indebted to the bacterial activities of the soil for the nitrogen which it passes on to the animal in elaborated form, so the growth vitamines which the animal obtains from the plant are not entirely manufactured by the plant as such, but are, at least partially, the products of bacterial activity in the soil in which the plants are growing.

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* Williams and Seidell, 'Jour. Biol. Chem.,' vol. 26, pp. 431–456 (1916).

† Livingston, B. E., U.S. Dept. Agric., Bureau of Soils, Bull. 36, 1907.

Summary.

1. Raw peat, when further decomposed by means of aërobic soil organisms —"bacterised peat"—is found to contain certain growth-promoting substances (auximones).

2. *Lemna minor* plants cannot maintain growth for any length of time in culture solutions containing only mineral nutrients.

3. The presence of soluble organic matter is essential for complete growth.

4. The addition to the mineral culture solution of 368 parts per million of organic matter from the water extract of bacterised peat resulted, after six weeks, in a multiplication of the number to 20 times, and an increase in weight to 62 times, that of the control plants. The water extract free from humic acid, representing an addition of 97 parts of organic matter per million, gave $9\frac{1}{2}$ times the number and 29 times the weight; 32 parts per million from the alcoholic extract gave $3\frac{1}{3}$ times the number and $7\frac{1}{3}$ times the weight; 13 parts per million from the phosphotungstic fraction gave $1\frac{1}{2}$ times the number and $2\frac{1}{2}$ times the weight.

5. The effect of the reduction in amount of auximones with successive fractionation of the bacterised peat was also manifest from the general appearance of the plants. Those in mineral nutrients only decreased in size

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* Brown, H. T., 'Trans. Guinness Research Lab.,' vol. 1, p. 288 *et seq.* (1906).

week by week, and became very unhealthy in appearance, whilst there was a progressive improvement in the appearance of the plants supplied with increasing amounts of auximones. Those receiving the larger amounts retained their normal healthy appearance throughout the experiment and increased in size.

6. The beneficial effect of the auximones was not due to a neutralisation of the toxic substances present in the ordinary distilled water, since comparable results were obtained with conductivity water.

7. An interchange of culture solutions, with and without auximones, showed that the plants are very sensitive to the presence or absence of these substances.

8. It is suggested that some of these growth-promoting substances may act directly as organic nutrients, and others may be of the nature of accessory food substances.

I am indebted to the authorities of the Botanical Department, Imperial College of Science and Technology, South Kensington, for facilities afforded for carrying out a considerable portion of the work in their greenhouse laboratory. I wish also to express my grateful thanks to my assistant, Miss Mockeridge, B.Sc. Without her valuable help it would have been impossible to obtain a record of the numbers and weights of the plants each week.

An Application of the Theory of Probabilities to the Study of a priori *Pathometry.*—Part II.

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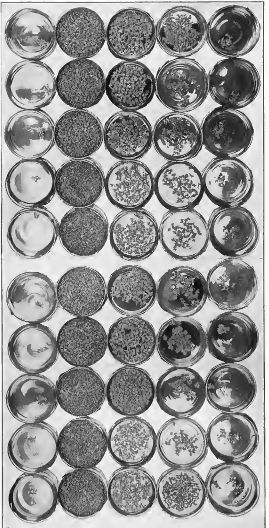
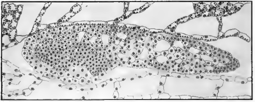
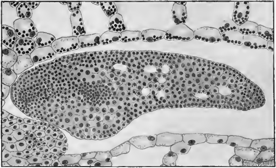

Series I
II
III
IV
V

Section through young frond. Series VI. × 235.



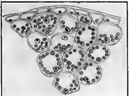
– Auximones.

Section through young frond. Series VIII. × 235.



+ Auximones.

Typical cells from old frond. Series VI.
× 610.



– Auximones.

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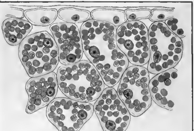
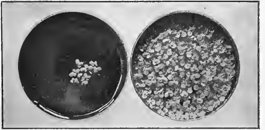


+Auximones.

PERIOD I.

Series I. Series II.



– Auximones. + Auximones.

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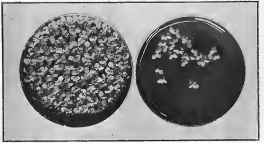

+ Auximones. – Auximones.

FIG. 6.